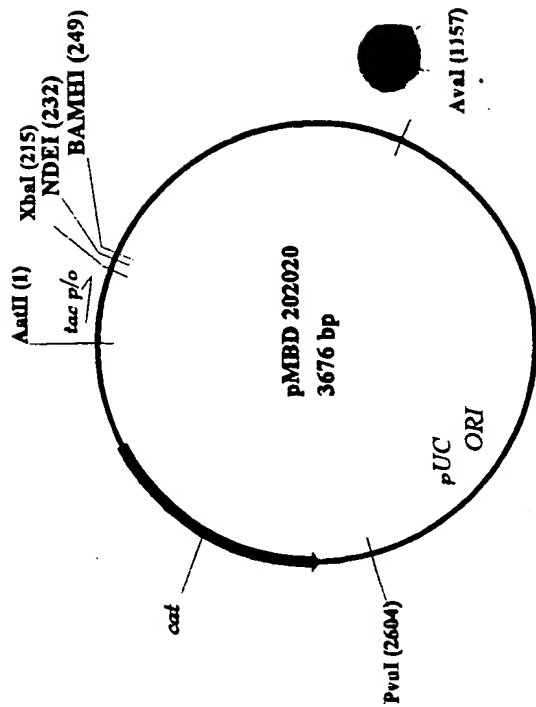


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**DIGEST
PCR FRAGMENT
AND
pMBD202020
NdeI/BamHI
LIGATE**

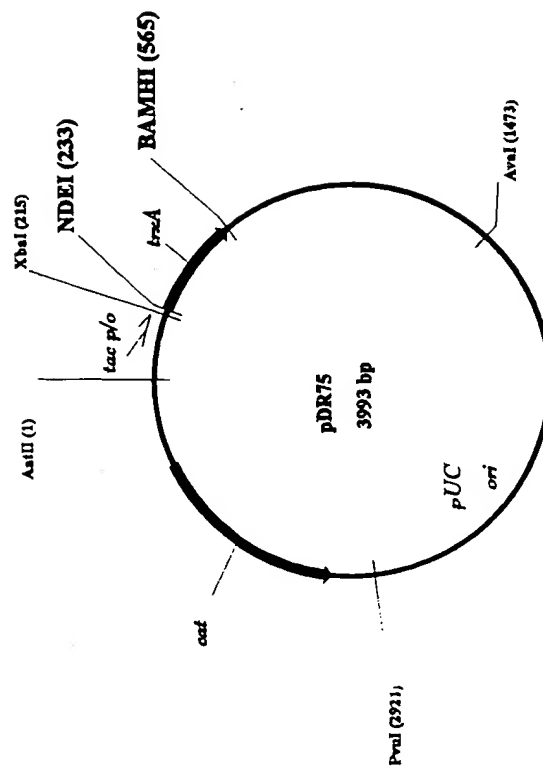
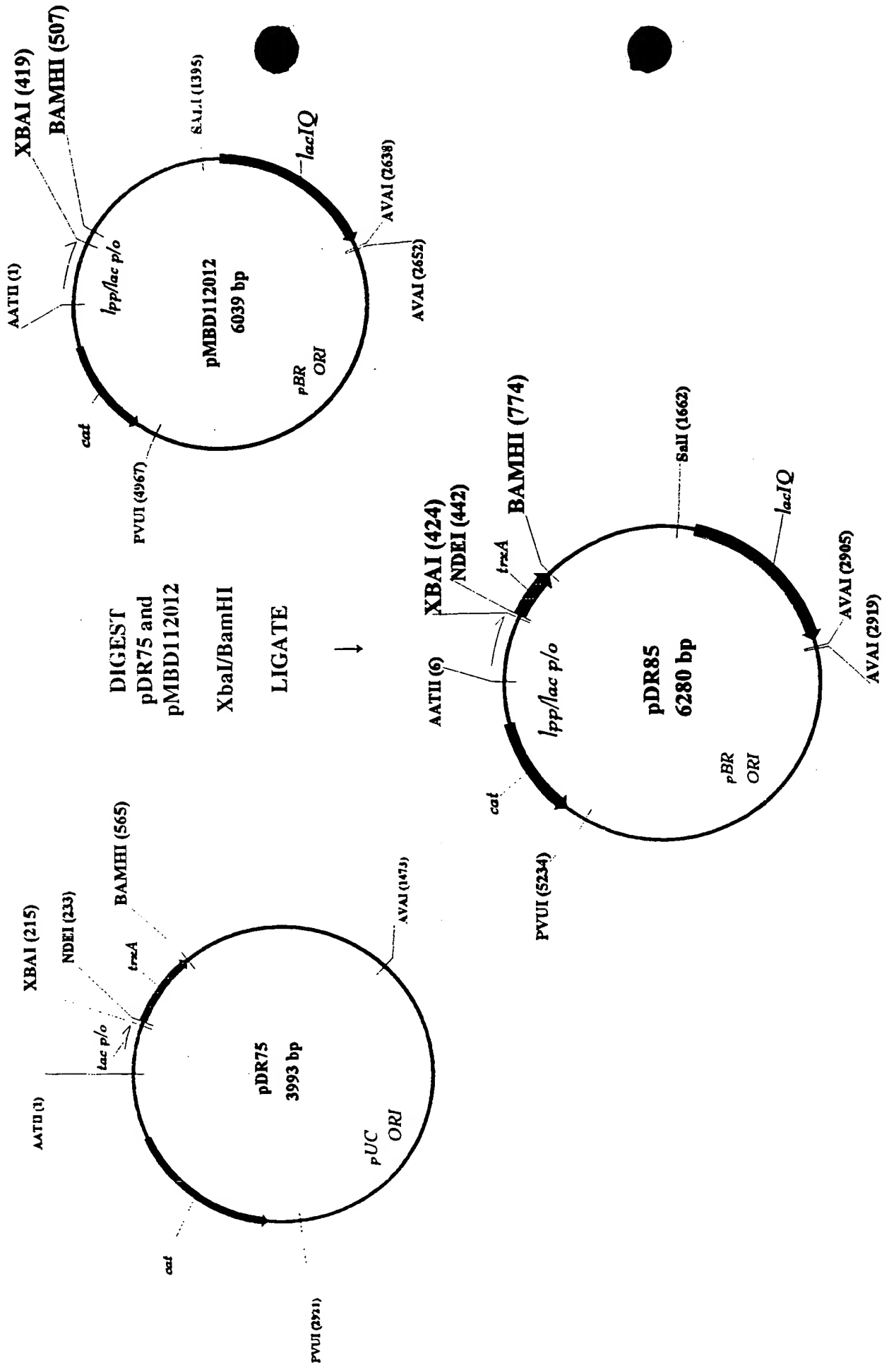
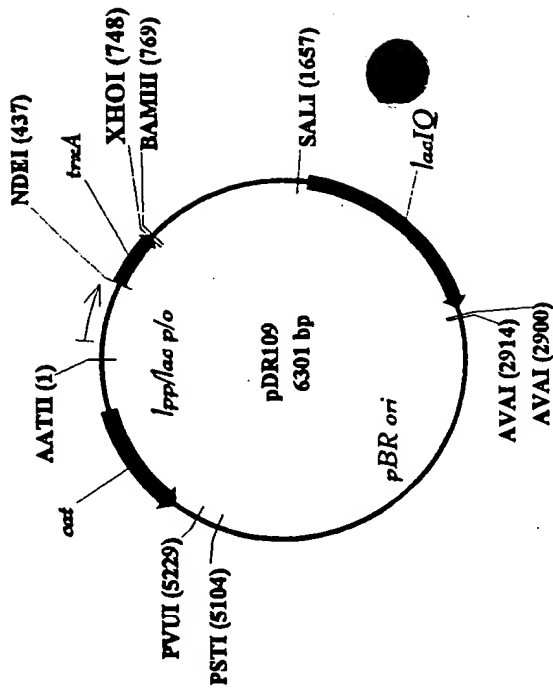
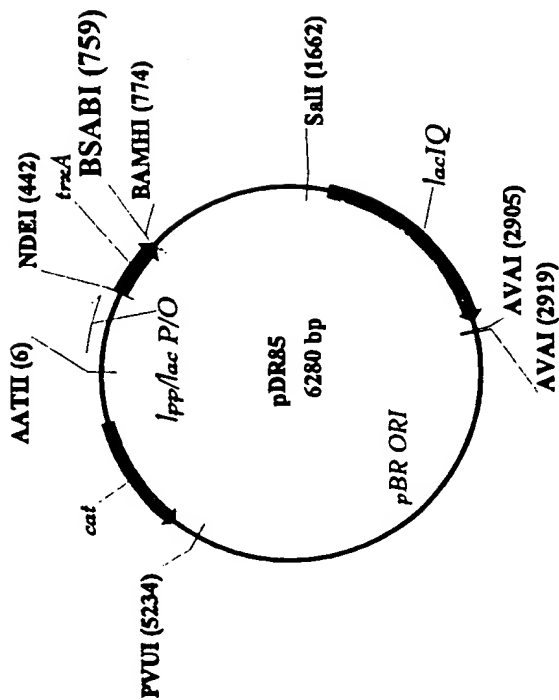


Figure 2
Schematic





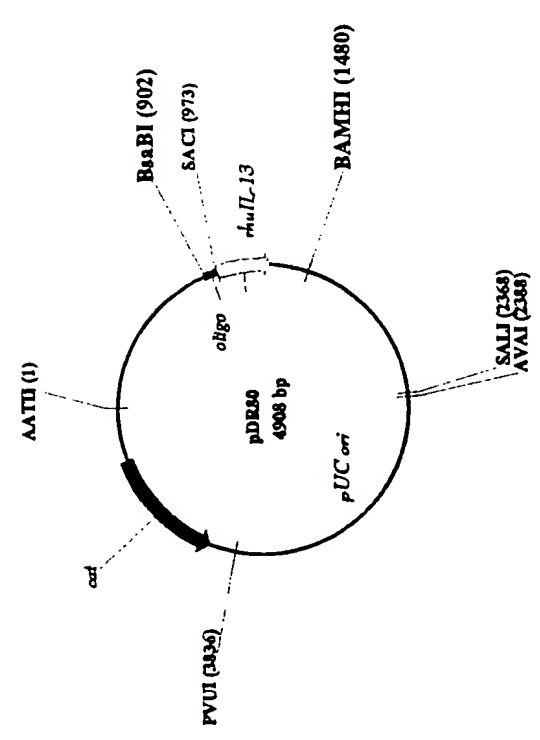
IN THE CONSTRUCTION OF pDR109 THE *trxA* GENE WAS ALTERED TO REPLACE THE BSABI SITE WITH A UNIQUE XHOI RESTRICTION SITE FOR EASY SUBCLONING OF A DOWNSTREAM RECOMBINANT GENE.

Leu Asp Ala Asn Leu Ala ***
 CTC GAT GCT AAT CTG GCG TAA
 ||| ||| ||| ||| ||| ||| |||
 CTC GAG GCT AAT CTG GCG TAA
 Leu Glu Ala Asn Leu Ala ***

pDR85 Sequence

pDR109 Sequence

Figure 4

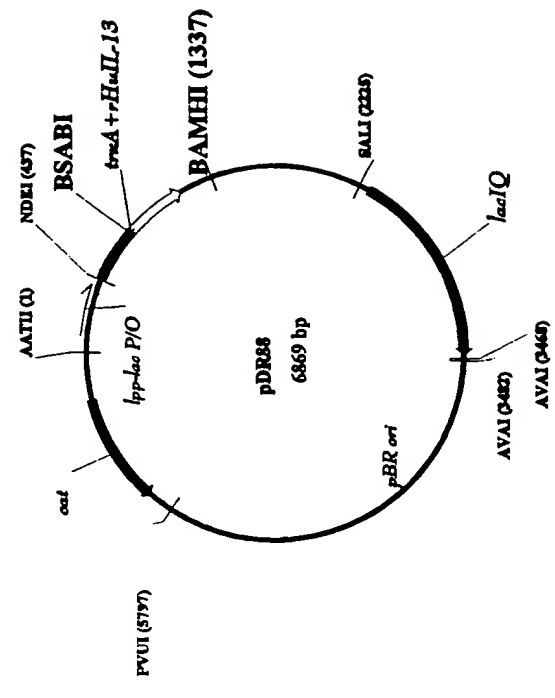
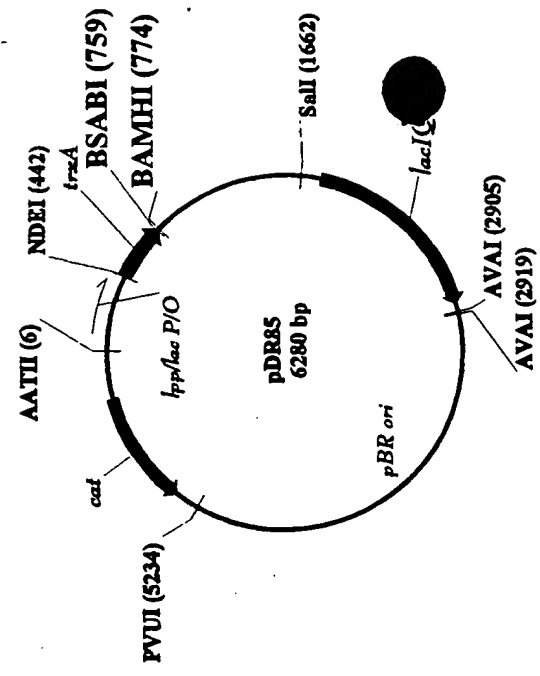


DIGEST
pDR80 & pDR85
BsaBI/BamHI

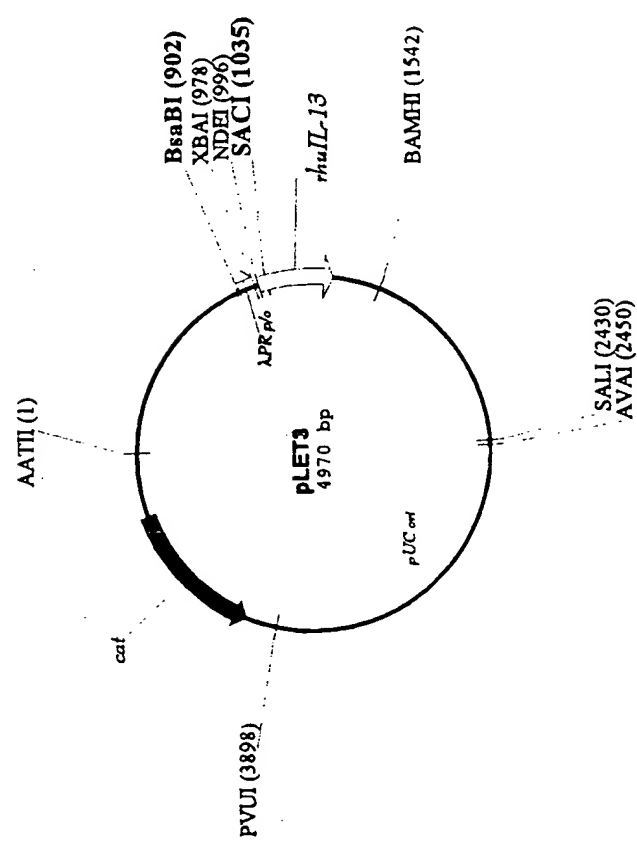
↓

LIGATE

↓



26 Oct 93 Figure 5



BsaBI-SacI oligo sequence			
GATAAATATCTGGCT	GGTCTGGTTCTGGT	GATGACGATGACAAG	
trxA sequence	glyserglysergly	aspaaspasp	lys
	linker region	enterokinase	cleavage
GGTCC	TGTTCCGCCG	TCCGCTCTGC	GTGAGCTC
rhuIL-13 sequence			

Synthesize both DNA strands of the BsaBI-SacI oligo
Digest pLET3 with BsaBI-SacI



Ligate pLET3 and the Oligo

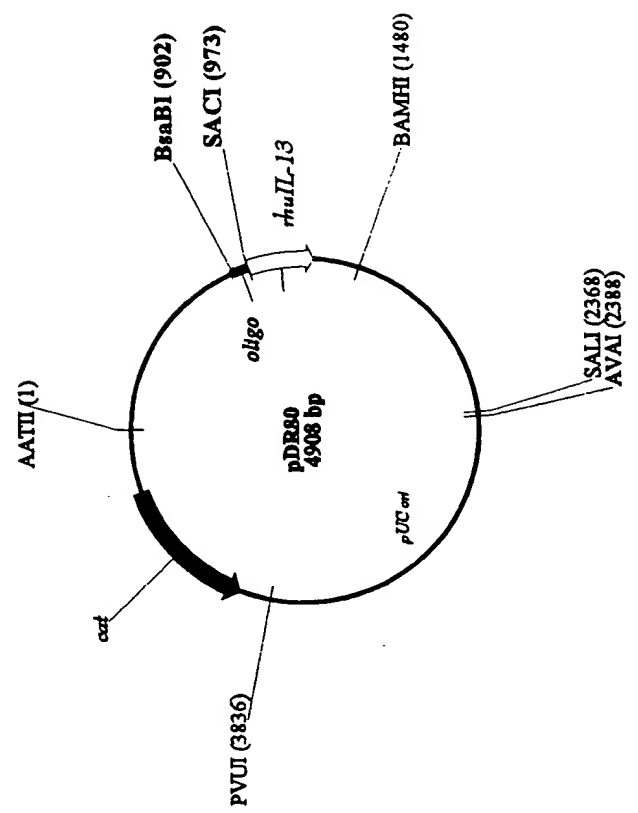
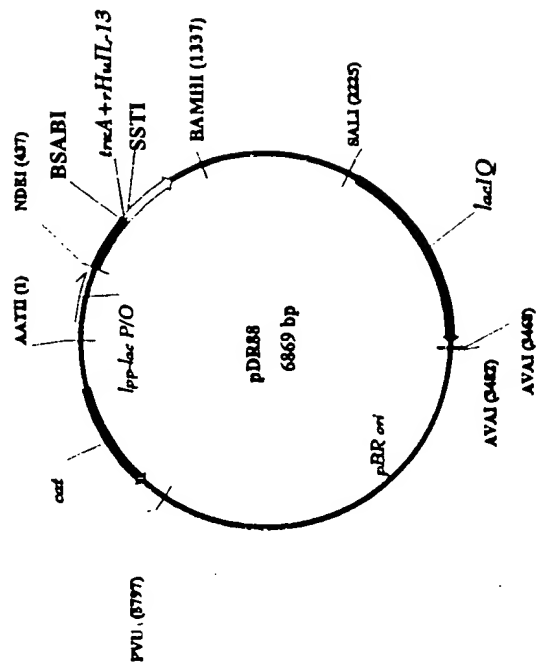
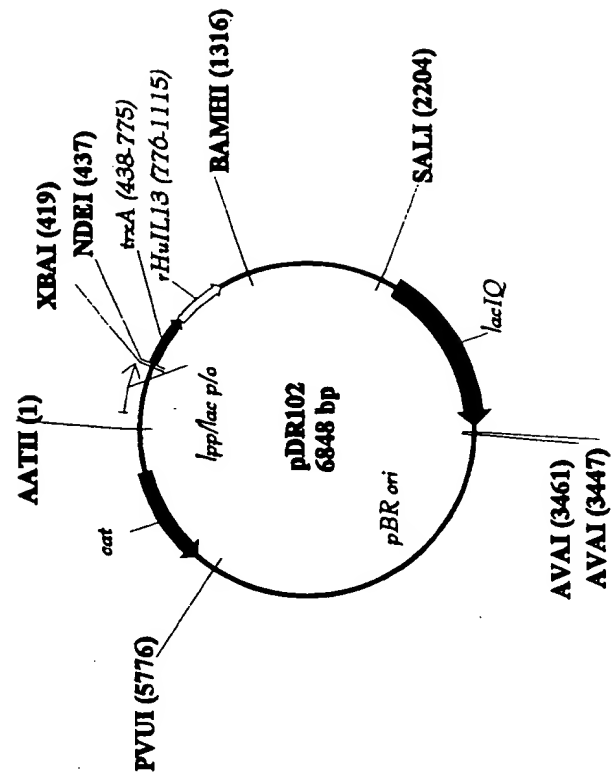


Figure 6



SYNTHESIZE A DOUBLE STRANDED OLIGO
WITH BSABI/SSTI RESTRICTION SITES

LIGATE INTO BSABI/SSTI DIGESTED pDR88



<u>R.B.S.</u>	<u>rhulL-13</u>	<u>SstI</u>
CCG AAG GAG GCT GAT TAAATG GGT CCG GTT CCG CCG TCT ACC GCT CTG CAG CTC		
	trxA	
	stop	

Figure 7

